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Effect of the Mitochondria-Targeted Antioxidant SkQ1 on Development of Spontaneous Tumors in BALB/c Mice

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Abstract—The mitochondria-targeted antioxidant SkQ1 (10-(6′-plastoquinonyldecyl)triphenylphosphonium) is a new pharmaceutical substance with a wide spectrum of effects including increase in lifespan of laboratory animals (for example, of BALB/c mice males) and inhibition of development of some experimental tumors and also of tumor cell growth. In this work, the effects of SkQ1 on development of spontaneous tumors in female and male BALB/c mice housed in an SPF-class vivarium were studied. We found that the addition of SkQ1 to drinking water at the dose of 1 and 30 nmol/kg body weight per day throughout the lifespan modified the spectrum of spontaneous tumors in the female mice, decreasing the incidence of follicular lymphomas. SkQ1 at the dose of 1 nmol/kg per day also suppressed the dissemination of these neoplasms, but it did not significantly influence the overall incidence of benign and malignant tumors (including primary multiple tumors) or the lifespan of the tumor-bearing mice (both males and females). Hence, the previously described ability of SkQ1 to increase the lifespan of laboratory BALB/c mice is not related to its anticarcinogenic activity.

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Key words: mitochondria-targeted antioxidants, SkQ1, spontaneous tumors, BALB/c mice

There is a widely held opinion that production of free radicals, mainly of mitochondrial origin, is one of the essential participants in pathogenesis of various diseases including carcinogenesis [1]. Based on this concept, some years ago a new class of pharmaceutical substances was developed – mitochondria-targeted antioxidants. Among them 10-(6′-plastoquinonyldecyl)triphenylphosphonium (SkQ1) is the best studied. It is important that various positive effects of this compound are manifested at very low (nanomolar) doses [2-7]. Therefore, it is interesting to study the influence of SkQ1 on tumor growth, and not only within the framework of the protocol of obligatory safety testing of new pharmaceuticals, but as a potential anticarcinogenic agent. Some data concerning this problem have been already published. SkQ1 suppressed the development of lymphomas in mice with the gene *p53* deficiency and inhibited the growth of human colon HCT116/p53$^{-/-}$ xenografts in nude mice [8]. SkQ1 did not significantly change the growth of SiHa tumor xenografts (HPV-16-associated cancer of human uterine cervix), but it noticeably increased the lifespan of tumor-bearing mice [7]. SkQ1 inhibited the development of benz(a)pyrene-induced soft tissue sarcomas [9]. However, SkQ1 did not significantly influence the development of spontaneous tumors in outbred SHR mice, inbred mice of the 129/Sv line, or transgenic HER-2/neu mice [5]. Thus, data on the inhibitory effect of SkQ1 on tumor growth in different models are not uniform; moreover, data on spontaneous carcinogenesis in mice without genetic modifications are limited – such data exist only for development of tumors in females of one inbred and one outbred line. Nevertheless, it is known that the spectra of spontaneous tumors in mice are very different depending on their line and sex [10].

The purpose of this work was to study the influence of SkQ1 on the incidence and spectrum of spontaneous neoplasms in male and female BALB/c mice, which is one of the most widely studied inbred lines. Another goal was motivated by previously published data showing that SkQ1 significantly increased the lifespan of BALB/c males but not females under conditions of an SPF vivarium [6]. The BALB/c line mice are characterized by

Abbreviations: ALS, average lifespan (days); SPF, specific pathogen free.

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extremely high incidence of different hematopoietic tumors (lymphomas and leukemias) along with very low incidence of mammary gland tumors, and this significantly distinguishes this line from two lines studied earlier. Thus, there is also the question whether the influence of SkQ1 on the development of spontaneous tumors plays a role in its geroprotective effect on BALB/c.

MATERIALS AND METHODS

Animals. BALB/c mice free of specific pathogenic and conditionally pathogenic microflora (from the Pushchino nursery) were kept under conditions of the SPF vivarium of the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (IBCh), Russian Academy of Sciences, until their natural death. The animals were kept at temperature 22°C with standard diet and water given *ad libitum*. The experiment was designed and the maintenance conditions conformed to the protocols established by the Bioethics Commission of IBCh.

SkQ1 treatment. The SkQ1 was synthesized according to the protocol published in the Supplement to works [2-4, 8]. The mice received the mitochondria-targeted antioxidant SkQ1 *per os* with water at the calculated dose 1 and 30 nmol/kg per day during their entire lifespan. The control animals received pure drinking water.

Necropsy and histopathological examination. All died animals were subjected to necropsy; however, only animals at the age of the appearance of tumors, whose corpses were suitable for macro- and microscopic diagnosis of tumors, were used for analysis of the incidence and spectrum of spontaneous neoplasms. Altogether 186 females and 105 males were analyzed (the control group included 62 females and 30 males), the group receiving 1 nmol SkQ1/kg per day including 59 females and 39 males, and the group receiving 30 nmol SkQ1/kg per day including 65 females and 36 males. During the necropsy all changes were recorded, and weights of the standard set of organs (the heart, liver, lungs, kidneys, brain) were determined. The brain, heart, spleen, mesenteric lymph node, both kidneys (with adrenals), a fragment of the liver middle lobe, lungs, and all organs with macroscopic lesions were examined histologically. The specimens were fixed with 10% formalin (pH 7.4), dehydrated with absolute isopropanol, and paraffin-embedded. Sections with thickness of 4 μm were stained with hematoxylin and eosin and if necessary by other methods (by Giemsa, Foot, van Gieson, PTAH (phosphotungstic acid–hematoxylin), PAS (periodic acid–Schiff)). Detected tumors were diagnosed and classified according to international recommendations [11, 12].

Analysis of results. In addition to determination of localization and histological type of the tumors, age (days) at death from different types of tumors, the dissemination of tumors (generalized and localized forms, *id est* for lymphomas – localized only in the spleen or in one lymph node), and cases of primary multiple tumors (hemangiomas and lung adenomas) were recorded. The results were evaluated statistically using Student's, Kruskal–Wallis, and χ^2-tests with SAS software.

RESULTS AND DISCUSSION

The total incidence of malignant tumors was the same in all groups in both males (control – 47%, 1 nmol/kg per day – 56%, 30 nmol/kg per day – 56%) and females (control – 92%, 1 nmol/kg per day – 92%, 30 nmol/kg per day – 92%) ($p > 0.1$). The average lifespan of the males with malignant tumors was slightly less than that of the females and was not changed under the influence of SkQ1 (see table). The overwhelming majority of tumors in the mice of all groups were histologically different types of hematopoietic neoplasms, bronchoalveolar adenomas, and pulmonary adenocarcinomas, and also vascular tumors – hemangiomas and hemangiosarcomas (of the liver, ovaries, spleen, adrenals, uterus, and soft tissues).

The hematopoietic neoplasms had the highest incidence in animals of all the groups. The females of all groups were characterized by especially high incidence of such tumors (84-89%), which was 2-4-fold higher than the incidence of lymphomas and leukemias in the males. Follicular and different variants of diffuse large cell lymphoma were prevalent, and lymphoblastic lymphoma was observed less often. There were also some cases of other tumors (splenic marginal zone lymphoma, small cell lymphoma, myeloid leukemia). In general, the total incidence of hematopoietic tumors in the males and females of the experimental groups did not significantly differ from the controls. However, in the females treated with SkQ1 at the daily dose of 1 and 30 nmol/kg a tendency was observed for a decrease in the incidence of follicular lymphoma (from 26 to 6 and 12%, $p = 0.053$ and 0.087, respectively). In the group treated with the daily dose of 1 nmol/kg, a significant decrease in the number of cases of disseminated follicular lymphoma was also elucidated (from 21% in the control to 6%, $p = 0.029$). The average lifespan of males and females with lymphomas and leukemias was approximately the same in all groups. The weight of the spleen that was taken as an additional parameter of tumor progression in the mice with lymphomas and leukemias did not differ in the animals of different groups.

Lung tumors in the BALB/c mice were relatively frequent (20-24%) as benign adenomas and malignant adenocarcinomas. Their incidence was not significantly different in the experimental and control animals. The lifespan of the animals with these tumors and the incidence of primary-multiple lung adenomas also did not differ. The lung involvement from adenocarcinomas estimated

Incidence of different types of spontaneous tumors and average lifespan (ALS) of control and SkQ1-treated tumor-bearing BALB/c mice

Parameter/group	Females			Males		
	control (n = 62)	SkQ1 daily, 1 nmol/kg (n = 59)	SkQ1 daily, 30 nmol/kg (n = 65)	control (n = 30)	SkQ1 daily, 1 nmol/kg (n = 39)	SkQ1 daily, 30 nmol/kg (n = 36)
Animals with malignant tumors, %	92	92	92	47	56	56
ALS of animals with malignant tumors	802 ± 102	785 ± 106	808 ± 94	742 ± 92	785 ± 82	762 ± 90
Animals with the hematopoietic tumors, %	89	88	84	23	41	39
ALS of animals with the hematopoietic tumors	799 ± 101	786 ± 106	801 ± 90	730 ± 124	800 ± 80	753 ± 86
Animals with follicular lymphomas, %	26	10*	12*	7	6	13
Animals with disseminated follicular lymphomas, %	21	6**	10	7	6	9
Animals with benign pulmonary adenomas, %	11	14	12	7	10	17
Animals with primary multiple pulmonary adenomas, %	2	2	2	3	5	3
ALS of animals with pulmonary adenomas	873 ± 118	881 ± 117	871 ± 98	757 ± 33	815 ± 39	785 ± 110
Animals with pulmonary adenocarcinomas, %	13	9	11	13	13	6
ALS of animals with pulmonary adenocarcinomas	853 ± 117	879 ± 98	861 ± 123	714 ± 44	747 ± 90	901 ± 69
Animals with benign hemangiomas, %	16	12	12	13	5	6
Animals with primary multiple hemangiomas, %	2	0	0	3	3	0
ALS of animals with hemangiomas	845 ± 73	841 ± 100	887 ± 88	799 ± 149	808 ± 30	748 ± 118
Animals with hemangiosarcomas, %	2	2	2	10	0	8
ALS of animals with hemangiosarcomas	922	699	808	784 ± 45	–	716 ± 63

Note: ALS is shown in days (M ± SD); parameters significantly different from the controls are indicated with asterisks: * $p < 0.1$; ** $p < 0.05$; in other cases the effect of SkQ1 was statistically insignificant.

by their weight was not different in the control mice and those treated with SkQ1.

BALB/c mice are characterized by a high incidence of tumors of endothelial origin (hemangiomas and hemangiosarcomas). The observed incidence of benign and malignant vascular neoplasms, including primary-multiple ones, did not significantly depend on SkQ1. The age of animals that died with these neoplasms was also the same.

In the mice of all groups and both sexes, fusiform (type A) or polygonal (type B) subcapsular hyperplasia of the adrenal cortex was observed. Moreover, single cases were recorded of spindle cell and pleomorphic cell soft-tissue sarcomas (rhabdomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma), histiocytic sarcoma, mesothelioma, cortical carcinoma of adrenals, hepatocarcinoma, salivary gland myoepithelial carcinoma, mammary gland carcinoma (type B), cecal carcinoid, squamous cell carcinoma of forestomach, Zymbal's gland and urinary bladder, leiomyoma and stromal polyp of uterus, and glandular polyp of stomach. These neoplasms were sporadic, and therefore it was impossible to conclude whether they are associated with the action of SkQ1.

Thus, in our experiments on male and female BALB/c mice SkQ1 did not significantly influence the total frequency of tumors or incidence of the majority of benign and malignant neoplasms. However, SkQ1 decreased the frequency of follicular lymphomas in the females and inhibited their dissemination, but it did not influence the lifespan of the animals with different types of tumors. Thus, we conclude that the ability of SkQ1 to increase the lifespan of BALB/c male mice found earlier [6] could not be caused by its anticarcinogenic activity. Moreover, this study additionally confirmed that SkQ1 does not have carcinogenic or tumor-promoting effects [5].

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